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United States Department of Agriculture Bureau of Entomology and Plant Quarantine

A REARING METHOD FOR THE MASS PRODUCTION OF MICROPLECTRON FUSCIPENNIS (ZETT.), COCOON PARASITE OF THE EUROPEAN SPRUCE SAWFLY, GILPINIA POLYTOMA (HTG.)

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#### INTRODUCTION

The purpose of this paper is to present a detailed description of a method which, it is believed, may be readily adapted to the large-scale production of small cocoon parasites similar in habit to <u>Microplectron fuscipennis</u> (Zett.). Excellent results have been obtained by the use of the apparatus and method described. The degree of parasitization has ranged from 20 to 90 percent, with an average of approximately 50 percent, and over 30 million <u>Microplectron</u> have been reared (for liberation) from approximately 3 million cocoons of <u>Gilpinia polytoma</u> (Htg.). As a result of liberations of this parasite, <u>Microplectron</u> has become established at many colony sites throughout the Northeast in the United States and in Southeastern Canada.

Microplectron fuscipennis is a gregarious chalcidoid parasite of sawfly cocoons and is polyphagous in habit. It has been reared in Europe from field-collected cocoons of such species of sawflies as Diprion pini (L.), Neodiprion sertifer (Geoff.), Gilpinia pallida (Klug), and Gilpinia polytoma. In North America Microplectron has been recovered from field collections of Gilpinia polytoma, Gilpinia frutetorum (F.), and Lygaeonematus erichsonii (Htg.), and has been reared in the laboratory on Neodiprion lecontei (Fitch). It is believed that it might be desirable to liberate Microplectron in infestations of injurious native or introduced sawflies in different parts of the United States.

### DEVELOPMENT OF THE BULK REARING METHOD

Entomologists of the Canadian Government have reared and liberated many million <u>Microplectron fuscipennis</u> in their fight against the European spruce sawfly (<u>Gilpinia polytoma</u>). Following severe outbreaks of this pest in Vermont and New Hampshire in 1937,

it became desirable to colonize the parasite in these areas and infested areas in other States. The Canadian entomologists have developed and used a rearing technique known as the "vial method." Microplectron females are isolated in small vials with two sawfly cocoons in each vial. This method seemed impractical because of the tremendous amount of labor involved in handling the individual parasites and cocoons. Therefore attention was directed toward the development of a technique for the rearing of Microplectron in bulk.

Preliminary experiments at the New Haven laboratory of the Division of Forest Insect Investigations indicated that a satisfactory bulk rearing method could be developed, and during 1937 a half million Microplectron were reared in this manner. In 1938 the writer was employed by the Society for the Protection of New Hampshire Forests, and appointed a Collaborator by the Bureau of Entomology and Plant Quarantine, to improve the method and equipment and to rear 5 to 10 million parasites. During 1939 the program was continued through the cooperation of the Vermont Department of Agriculture and the New York Department of Conservation. Approximately 20 million Microplectron were reared and distributed throughout the New England States and New York. In addition to these liberations in Gilpinia polytoma infestations, 500,000 Microplectron were released in 1939 in New Jersey and 390,000 in Ohio in Neodiprion sertifer infestations.

# LIFE HISTORY OF MICROPLECTRON

Under laboratory conditions the life cycle of Microplectron is completed in 15 to 18 days and it is possible to rear 18 generations in a year. Mating takes place immediately after the emergence of the parasites from the cocoon. Oviposition in the cocoons is usually completed in 24 to 36 hours after emergence. The eggs hatch in 2 days, and larval development extends over a period of about 10 days. The prepupal and pupal period ranges from 3 to 6 days, depending upon the number of individuals present in the cocoon. Development takes longer when only a few individuals are present because these individuals are much larger than is the case when the cocoon is crowded. The adult parasites emerge through a tiny round hole cut near one end of the cocoon. Occasionally two holes will be found in the same cocoon, one at each end. The normal sex ratio of Microplectron is 1 male to 5 females, and in the rearing of this species it is important that females be allowed ample time in which to mate, since the progeny of unmated females are all males. The number of parasites per cocoon in the material reared in the laboratory ranges from 1 to 100, with an average of about 30. In the field the immature larvae of the last generation

Constant temperature approximately 76° F., constant humidity approximately 85 percent R. H.

in the fall spend the winter within the cocoon, completing their development the following spring. In laboratory tests, at temperatures approximating field conditions, adult females have lived 30 days.

## DESCRIPTION OF THE EQUIPMENT

## The Emergence and Exposure Boxes

Owing to the minute size of the <u>Microplectron</u> adults (from 0.5 mm. to 2.0 mm. in length) it is difficult to confine them in any type of screened cage. A solid box was designed and constructed on the principle of the Schmitt box, with a glass-topped removable cover (fig. 2). The glass fits snugly into slots in the cover frame, which is also provided with outer shoulders that slide firmly down over the slightly beveled inner shoulders of the body of the box. In the middle of one end of the box is a  $1\frac{3}{4}$ -inch, slightly tapered hole, which is fitted with a No. 23 cork. The sides of the box are grooved to receive the ends, and the bottom is tongued into the sides. This construction makes the box solid and insect-proof. To facilitate cleansing, the box is coated on the inside with high-melting-point paraffin. The same type of box is used as an emergence or as an exposure box. Details of construction and dimensions are indicated in figure 1.

## The Exposure Trays

Each exposure box is fitted with a set of five trays to hold exposed cocoons (fig. 3). The tray is a wooden frame one-half inch high and one-eighth inch smaller in width and length than the inner dimensions of the box. The bottom of the tray is a sheet of 12-mesh window screen. The lowest tray is raised from the floor of the box by means of four nails driven into the corners of the lower side of the tray. The construction of these trays is such that the attacking parasites have free access to each cocoon exposed.

#### The Corks

For the purpose of allowing migration of the parasites from an emergence box to an exposure box, a hollow double cork is provided (fig. 2, A). This arrangement is made by strapping two No. 23 corks back to back with tape and boring a 1-inch hole through the axis, thus forming a tube with tapering ends that will fit the holes in the ends of the exposure and emergence boxes. The boxes to be used for emergence are fitted with corks having a 1-inch ventilation hole covered with wire cloth (fig. 2, B). Solid corks (fig. 2, C) are used in the exposure boxes while they are in the incubating room, to retain the humidity.

#### REARING PROCEDURE

## Method of Storing Stock Cocoons

The European spruce sawfly cocoons used in the rearing work are collected in the fall after the second-generation cocoons have been formed. The cocoons are separated from the moss and other debris by screening and winnowing. If the cocoons are excessively wet, they are allowed to dry out somewhat before screening. When wet cocoons are put into refrigeration some mortality results from mold formation. The cleaned cocoons are placed in wooden-frame trays 12 inches square by 3 inches deep, the bottoms of which are covered with 12-mesh copper screen. A tray of the above dimensions will hold 35,000 cocoons. On each corner of each tray is a wooden block which keeps the trays apart when they are stacked in the refrigerator, thus allowing free circulation of air around each tray. Since the trays are filled with cocoons, a cloth is pasted over the top of each tray to prevent spilling of the cocoons when the trays are handled in refrigeration. Very satisfactory results are obtained by storing the cocoons in these trays at a temperature of approximately 33° F. and a relative humidity of approximately 96 percent.

## Method of Exposure

Microplectron are placed in a parasite emergence box. This box is kept in the incubation room until adult parasites begin to emerge. Meanwhile four exposure boxes are prepared. Stock cocoons from storage are placed one layer deep in the wire racks and on the floor of the box. About 3,000 cocoons can be placed in each box containing five trays. Feeding pads, made of small squares of absorbent cotton soaked in honey-water solution (20 percent honey), are placed on squares of waxed cardboard in alternate trays. The feeding pads provide a source of food and water for the incoming parasites and aid materially in maintaining the proper humidity in the exposure box.

When adult parasites begin to appear in the emergence box it is covered to shut out the light, and the box is then attached to an exposure box by means of a hollow cork migration tube. Since Microplectron adults respond positively to a light stimulus, they crawl through the tube into the exposure box. The number of parasites entering is easily estimated after a little experience. After approximately 5,000 parasites have entered the exposure box it is removed, corked, and placed in incubation at approximately 76° F. and 85 percent relative humidity. A second exposure box is then attached to the emergence box, and the process is continued until emergence is complete. On the twelfth day of incubation, when the parasite larvae are mature, the box is removed from the incubation room, and the cocoons are taken out, sampled, and put into storage

at 32° to 36° F. until needed for rearing or liberation. Development may be retarded for several months without injury to the parasites.

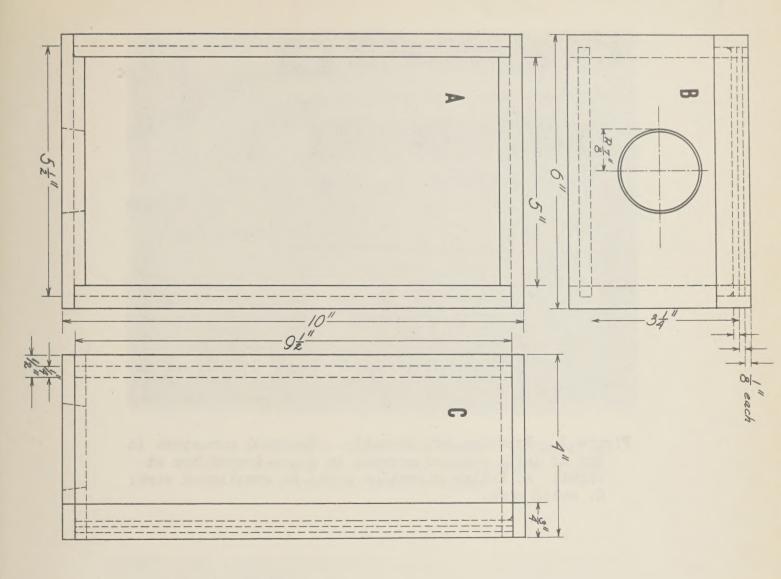
## Method of Sampling

A sample of the cocoons exposed on each date is taken at the completion of the incubation period. A random sample of 100 cocoons is taken from each box, and each of the cocoons is opened. Since Microplectron larvae are external feeders, the percentage of parasitized, normal, emerged, or dead sawflies is easily determined. This extensive sampling results in no waste, since the cocoons found to contain Microplectron pupae are used in the exposure boxes. The percentage of parasitization in the sample is taken as an indication of the degree of parasitization in the lot. Later, when the liberation frames are being prepared, the number of cocoons necessary to produce a given number of parasites is determined.

#### Method of Parasite Liberation

As a measure of precaution all liberations of Microplectron are made by confining the parasitized cocoons in a wooden frame 6 inches square and 1 inch high, covered on both sides with 12-mesh wire screen (fig. 4). In this manner Microplectron adults are allowed free exit, but any emerging sawfly adults are retained by the screen. The frame is made with one screen tacked in place. It is then filled with damp sphagnum moss with a layer of cocoons on the top and bottom. The second screen is then tacked in place and the information label is attached. The frames are carried to the liberation area in iced insulated packing cases. Each frame is placed on the north side of a tree and leaned against the base. The majority of the parasites are in the pupal stage and they mature and begin to emerge in about 3 days. Emergence is continuous over a period of 10 days, with the peak coming at about the sixth day. Each frame contains a sufficient number of parasitized cocoons to produce 10,000 Microplectron adults. The cardboard printed label attached to each frame explains its use and requests that it be not disturbed.

When liberations of <u>Microplectron</u> reared on spruce sawfly cocoons are made in areas where the European spruce sawfly is not known to occur, two precautions are observed. Before exposure to the parasites the sawfly larvae are paralyzed by a 3-minute immersion of the cocoons in a water bath at 58° C. (136.4° F.). After incubation of the parasites, each cocoon is pierced through the middle with a needle before it is placed in the liberation frame. In this manner parasites reared on the easily obtainable spruce sawfly cocoons may be liberated in areas infested by other sawflies, the cocoons of which are difficult to obtain in large numbers, without danger of introducing the spruce sawfly.



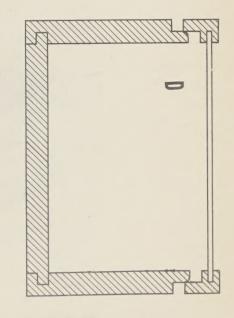


FIG. 1

REARING BOX

FOR SMALL COCOON

PARASITES

(ASSEMBLY DRAWINGS)

TOP VIEW OF BOX.

END VIEW OF BOX.

W

C SIDE VIEW OF BOX.

VERTICAL PLANE SECTION SHOWING COVER PARTLY REMOVED.

New Haven, Connecticut - Dec. 19, 1939
F. E. Miller, Ur.

Figure 1. -- Details of the rearing box assembly.

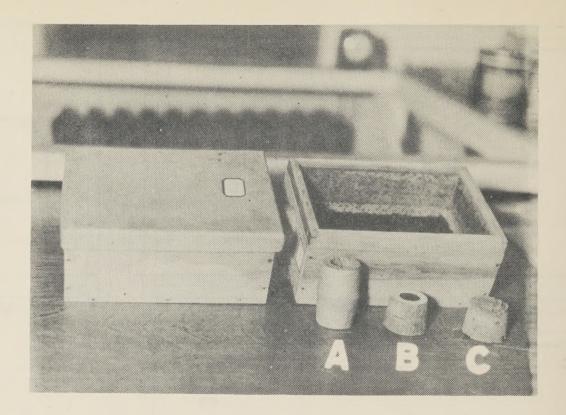


Figure 2.—Exposure box assembly. Emerging parasites in box at left; exposed cocoons in glass-topped box at right. A, Hollow migration cork; B, ventilated cork; C, solid cork.

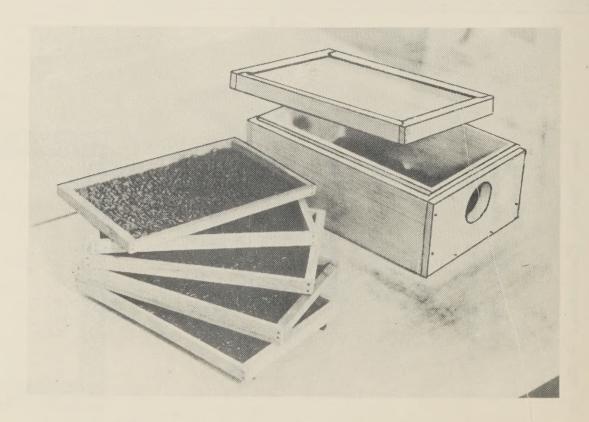


Figure 3.—Exposure box, showing screen trays of cocoons prepared for exposure.

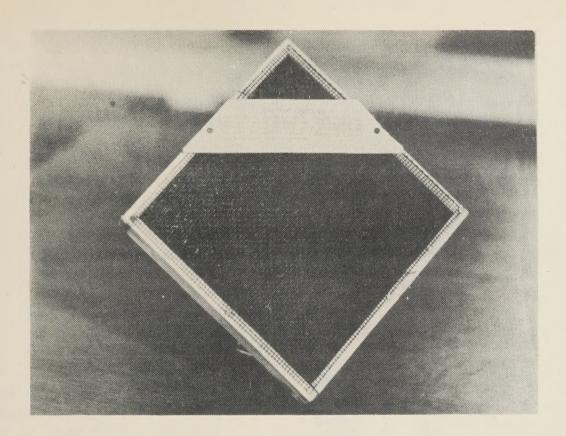


Figure 4.—Parasite liberation frame. Parasitized sawfly cocoons in a wooden, wire-covered frame, with an information label attached.

